Supplemental Information

Supplemental Figure S1 Direct immunofluorescence microscopy showing the transfection efficiency of EGFP-Src (Src) and Cherry-FRB (FRB), serving as input control for Fig. 1c.

Supplemental Figure S2 (**a)** Exogenous co-immunoprecipitation studies in HEK293 cells show that activation of Src-iFKBP (Src*) increases the interaction of Myc-synaptopodin (Synpo-WT) with FLAG-calcineurin A (FLAG-CnA) when compared with inactive Src-iFKBP (Src). Alanine substitution of Y29 (Synpo-Y29A) or Y344 (Synpo-Y344A) does not interfere with calcineurin binding at baseline (Src) or activation of Src (Src*). No interaction is found with the negative control Myc-Raver. In the FLAG eluate, FLAG-CnA is marked by an arrow, the other bands represent heavy (*) and light chain (**) of the anti-FLAG antibody that was used to precipitate FLAG-CnA. Molecular weight markers are in kDa. (**b**) Direct immunofluorescence microscopy showing the transfection efficiency of EGFP-Src (Src) and Cherry-FRB (FRB).

Supplemental Figure S3 Exogenous co-immunoprecipitation studies in HEK293 show that Src inhibitor 1 (SrcI-1) lowers the basal interaction between FLAG-calcineurin and Myc-synaptopodin. No interaction is found with the negative control Myc-Raver.

Supplemental Figure S4 (**a**) Immunofluorescence of EGFP-Src and Cherry-FRB shows near complete infection/expression efficiency in podocytes; scale bar 20 μm. (**b**) The calcineurin inhibitor CsA does not restore stress fibres in synaptopodin-depleted (synpo shRNA) cells in the presence of activated Src (Src*); scale bar 20 μm.

Supplemental Figure S5 (**a**) Phalloidin labelling reveals well-developed stress fibres in non-infected control podocytes before (left) and after rapamcyin treatment (middle) and in Src-iFKB/FRB co-infected podocytes (right); scale bar 20 μm. (**b**) Synaptopodin protein abundance is comparable between control (con) and rapamcyin (rapa) treated podocytes. GAPDH serves as a loading control. (**c**) Protein abundance of active pSrc (Y416), inactive pSrc (Y527) and total Src is comparable between control (con) and rapamcyin (rapa) treated podocytes. Molecular weight markers are in kDa. (**d**) Western blot analysis shows the degree of protein depletion in podocytes expressing shRNAs that target synaptopodin (Synpo), Vav2 or Rac1. GAPDH serves as a loading control.

Supplemental Figure S6 The Rac1 inhibitor NSC23766SF restores stress fibres in synaptopodin-depleted (synpo shRNA) cells; scale bar 20 μm.

Supplemental Figure S7 Lck mRNA expression is readily detected in spleen (positive control) but is absent from isolated mouse kidney glomeruli (glom), undifferentiated (undiff) or differentiated (diff) podocytes. In contrast, c-Src expression is found in glomeruli, undifferentiated and differentiated podocytes and in spleen. GAPDH serves as loading control.

Supplemental Figure S8 As a control experiment, phalloidin labelling reveals that overexpression of four different GFP-synaptopodin constructs has no effect on stress fibres in podocytes prior to PS treatment; scale bar 20 μm.

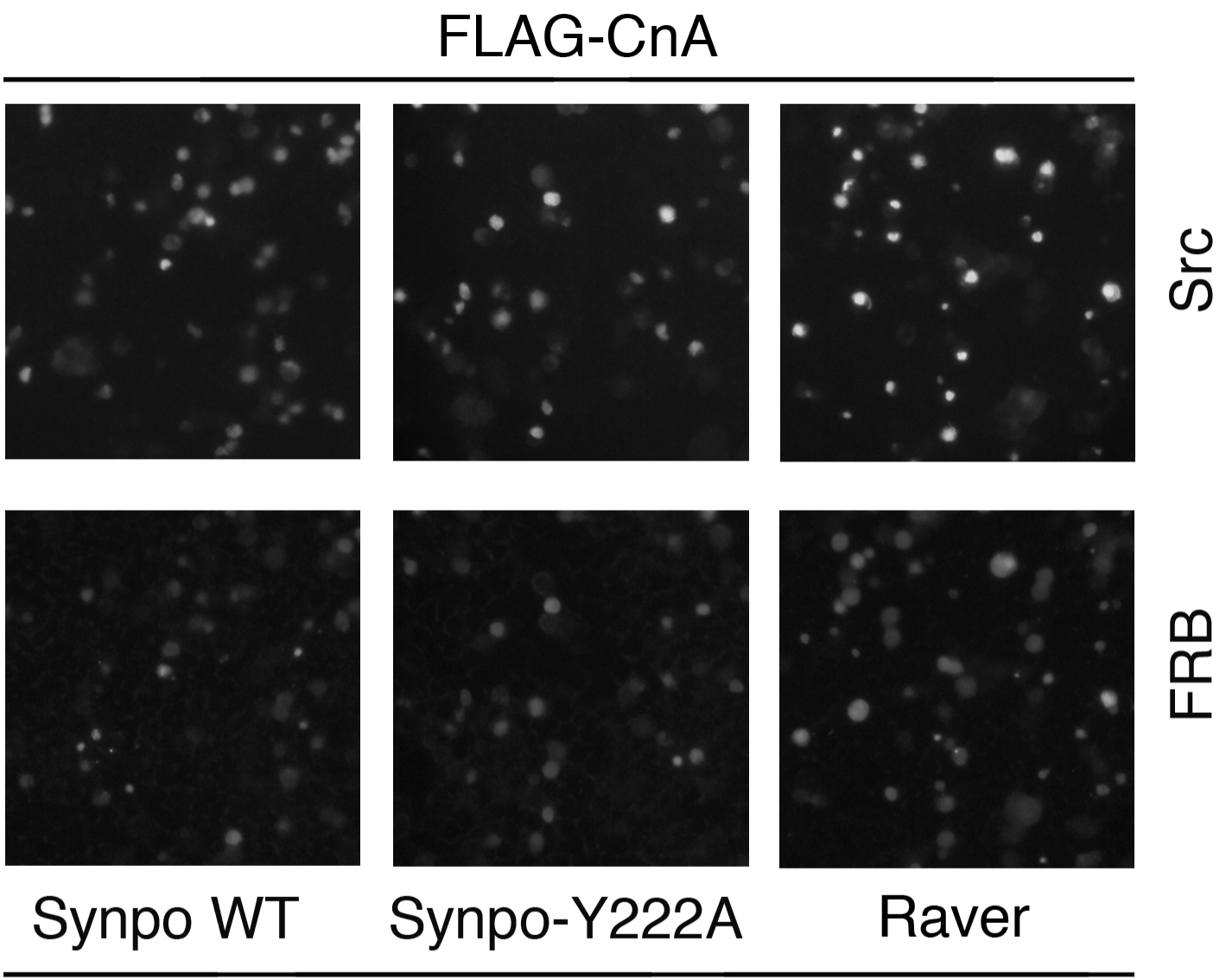
Supplemental Figure S9 As a control experiment, phalloidin labelling reveals that two independent shRNAs for synpo (#3 and #4) have the same effect on disrupting stress fibres in podocytes, which is rescued by the Rac1 inhibitor NSC27366; scale bar 20 μm.

Supplemental Table S1 Sequences of shRNAs used for gene silencing experiments

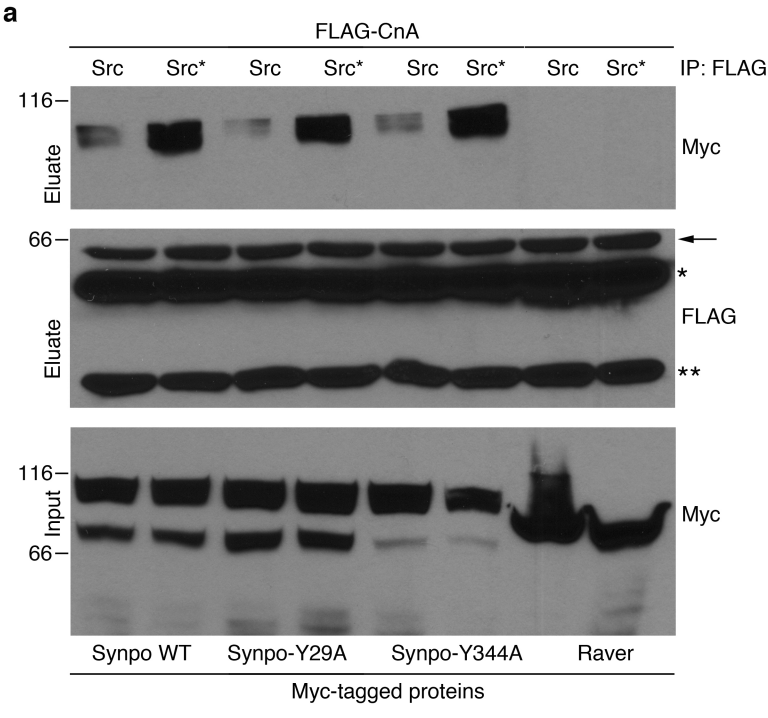
shRNA	Sequence
VAV2 1	CCGGAAGTGGAGGAGTGCACACTCACTCGAGTGAGTGTGCACTCCTCCACTTTTTTTG
VAV2 2	CCGGAAGGAATGCCTGGAGGTGATCCTCGAGGATCACCTCCAGGCATTCCTTTTTTTG
p190RhoGAP1	CCGGCGGTACATTAGAGATGCACATCTCGAGATGTGCATCTCTAATGTACCGT
	TTTTG
p190RhoGAP2	CCGGCGGTTGGTTCATGGGTACATTCTCGAGAATGTACCCATGAACCAACCGTTTTTG
Rac1	CCGGCGCAGACAGACGTGTTCTTAACTCGAGTTAAGAACACGTCTGTCTGCGTTTTTG

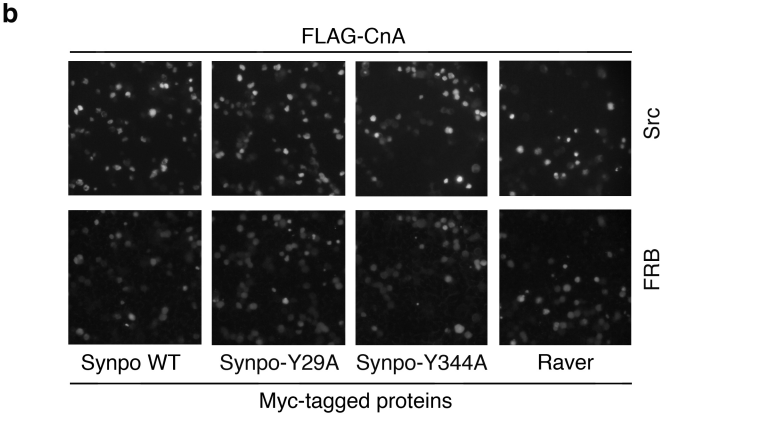
Supplemental Table S2 Sequences of RT PCR primers for mRNA Src kinase detection

Primer	
Src Forward	CGGCTGCAGATTGTCAATAA
Src Reverse	AGGCTTGGATGTGGGACATA
Lck Forward	ATTACATCCATCGGGACCTG
Lck Reverse	CATCCAGAACACTCCGAAGG
GAPDH Forward	CTGCACCACCAACTGCTTAGC
GAPDH Reverse	GGCATGGACTGTGGTCATGAG

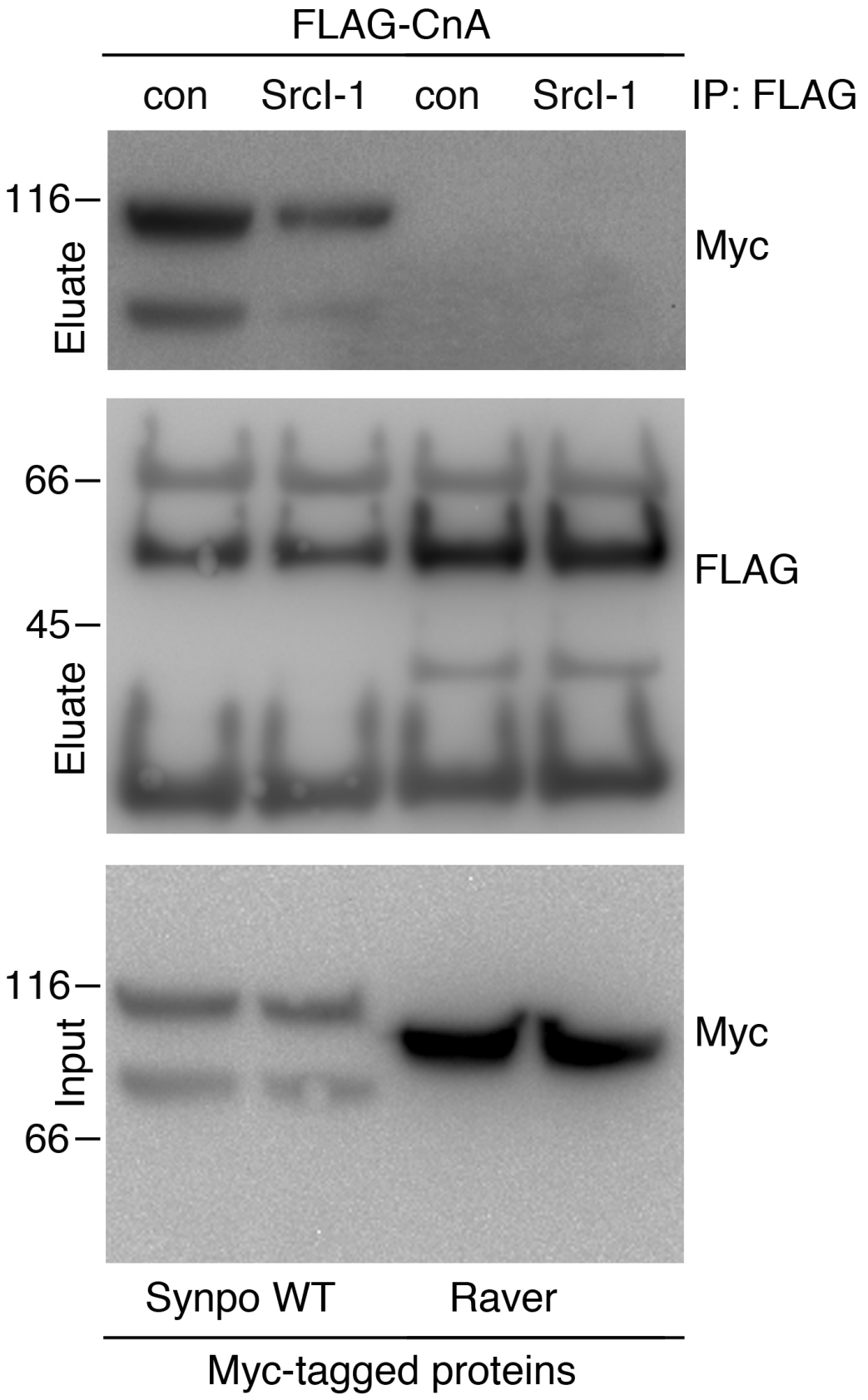


Supplemental Figure S1

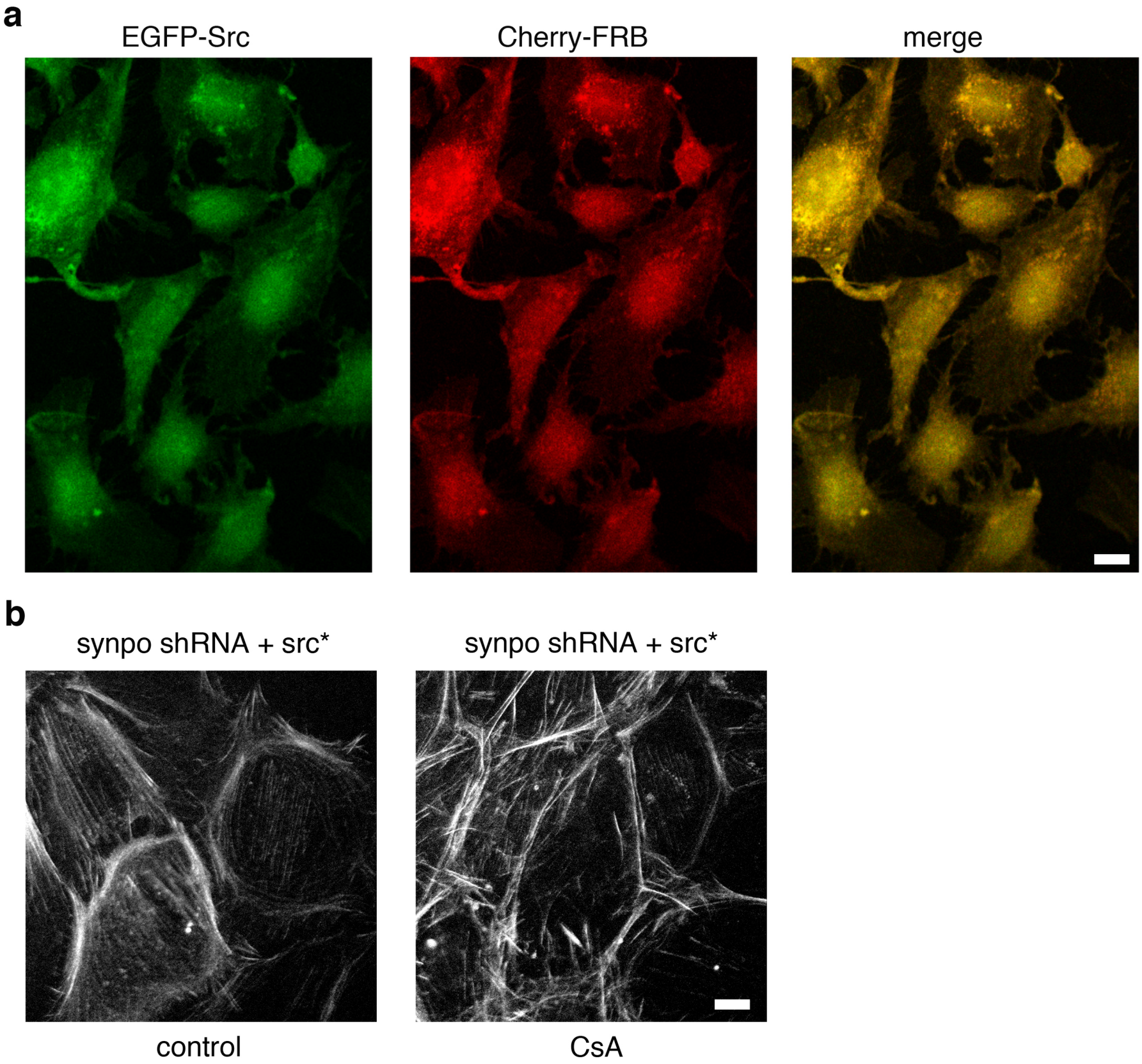




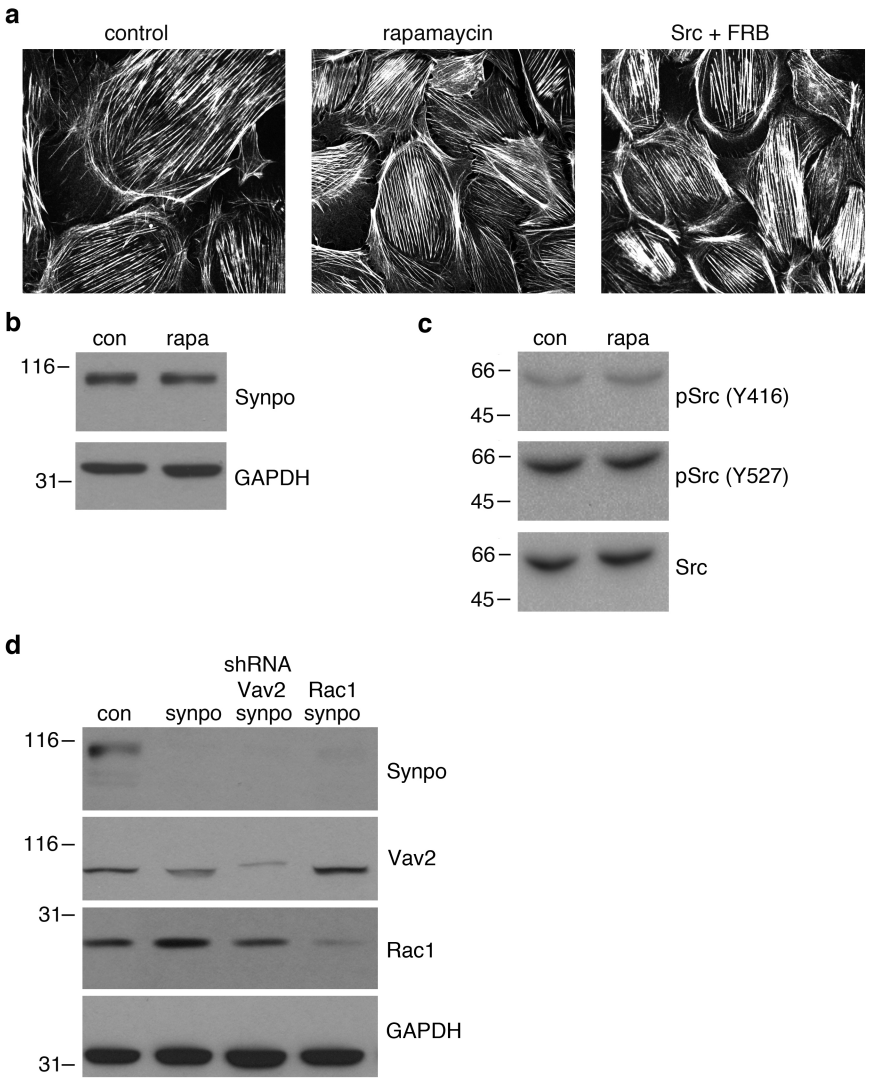
Supplemental Figure S2



Supplemental Figure S3

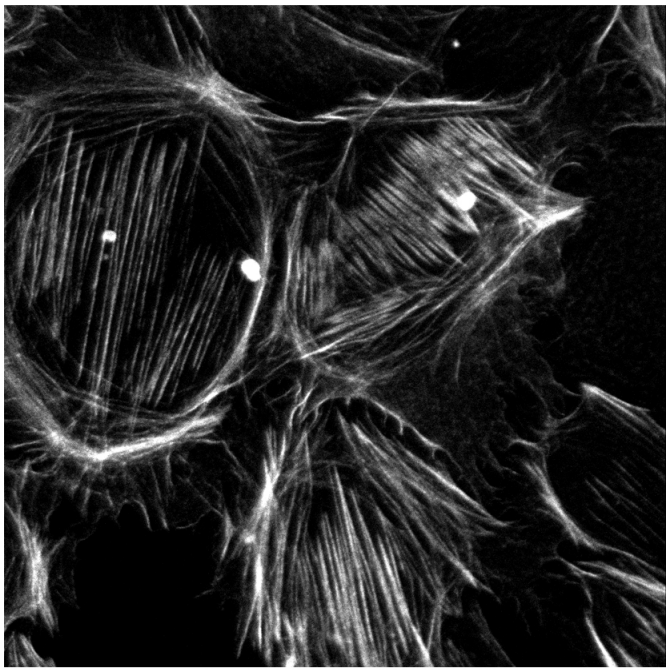


Supplemental Figure S4



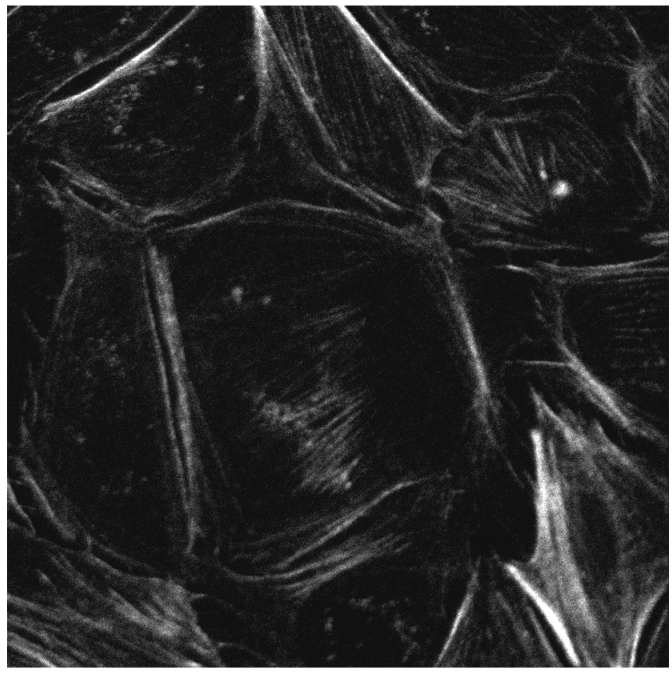
Supplemental Figure S5

control shRNA



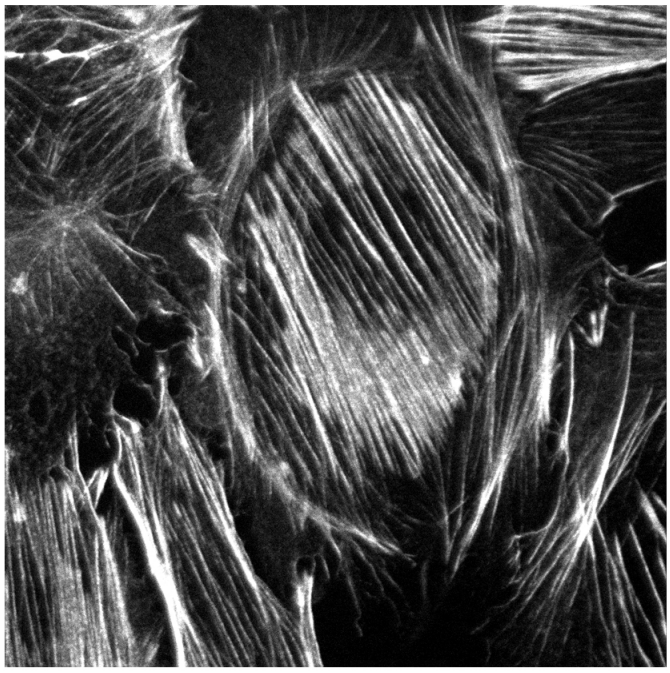
control

synpo shRNA



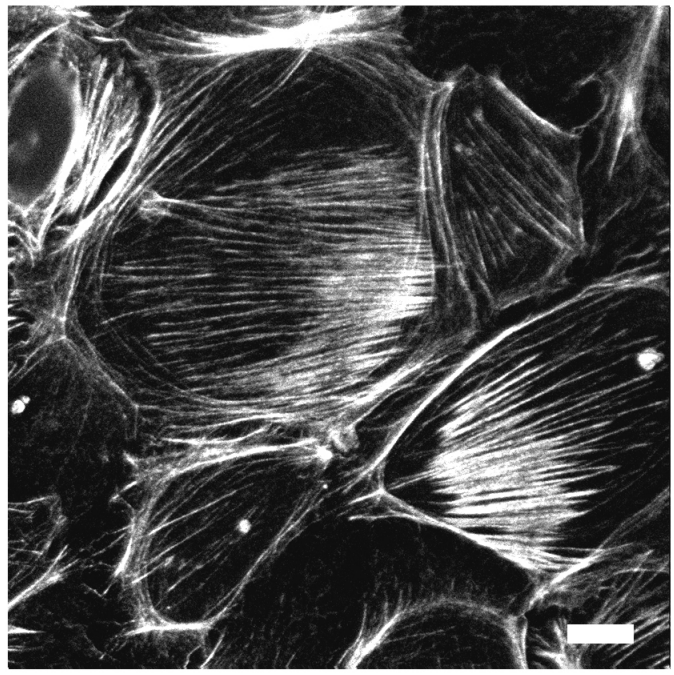
control

control shRNA



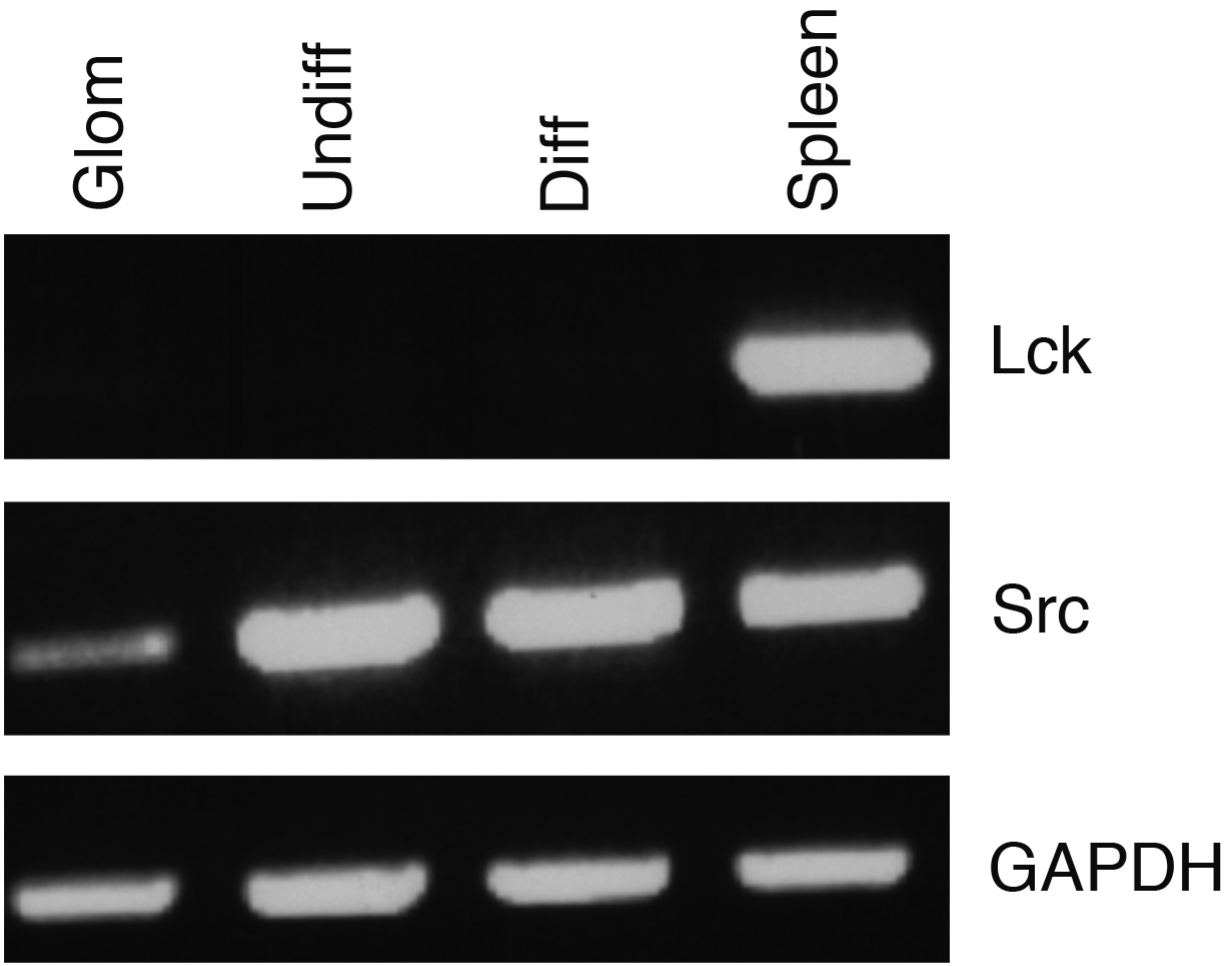
control

synpo shRNA

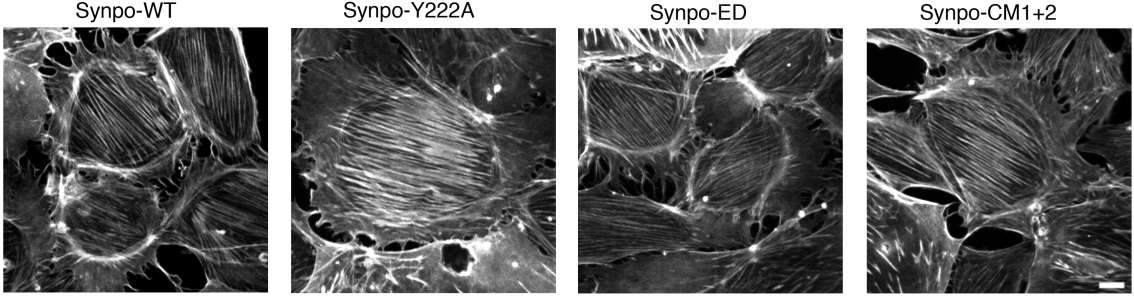


NSC27366

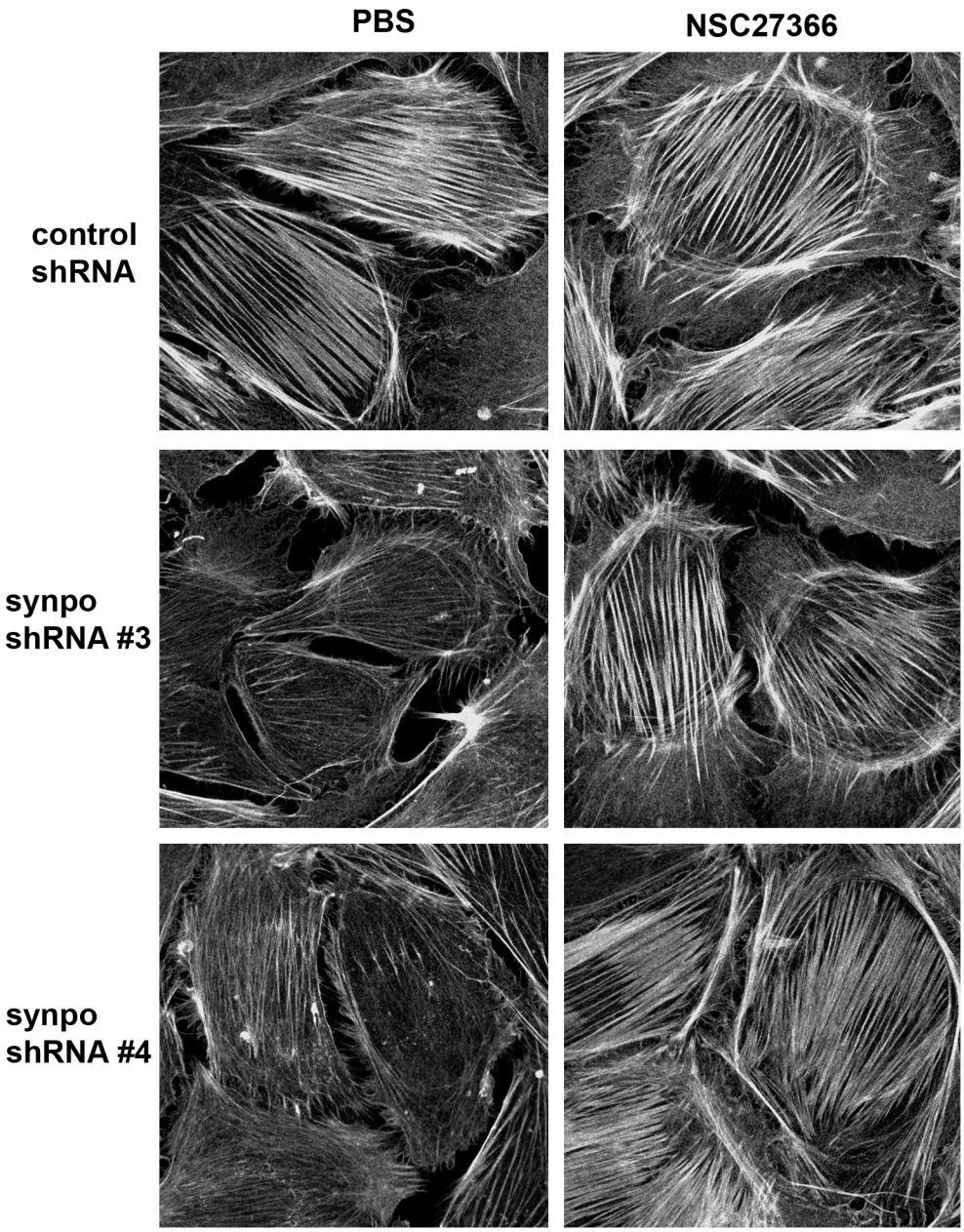
Supplemental Figure S6



Supplemental Figure S7



Supplemental Figure S8



Supplemental Figure 9